Vanishing white matter

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CHAPTER 7

Summary, discussion
and future perspectives

VANISHING WHITE MATTER DISEASE

Vanishing white matter disease[1] (VWM) is a puzzling leukoencephalopathy caused by mutations in any of the five genes encoding eukaryotic translation initiation factor 2B (eIF2B), an ubiquitously expressed protein complex with a crucial role in initiation of mRNA translation for virtually every protein in the human body.[2-5]

VWM is one of the most prevalent inherited childhood white matter disorders[6], but the disease may occur at all ages.[7,8] Most patients with VWM show signs of progressive neurologic deterioration with predominantly signs of motor dysfunction. In the past years it has become known that the clinical spectrum is extremely wide with, for example, migraines, psychiatric symptoms and presenile dementia as start of the disease at older age. In some patients the disease also affects other organs, especially the ovaries. Only in patients with the most severe variant of the disease, the antenatal onset variant, other organs can be affected as well.[7-12] The list of known VWM causing mutations is still expanding.[8,13]

The disease is fatal. There is no specific treatment for VWM. Management is at present supportive.[8]

Typical findings on MRI are symmetrical diffuse abnormality of the cerebral hemispheric white matter with rarefaction and cystic degeneration in a "melting-away" pattern.[1,7] Neuropathological findings show a cavitating white matter disease with meagre reactive gliosis and involvement of the glial lineage with a maturation defect of astrocytes and oligodendrocytes.[1,8,9,14-17]

The link between the selective vulnerability of the white matter of the brain and the mutated eIF2B protein complex is not understood.

The main goal of this thesis was to increase our understanding of the phenotypic variation and the correlation between genotype and phenotype in VWM.

CLINICAL VARIATION IN VWM

The disease onset, clinical severity and disease course of VWM patients vary greatly.[7-12] Large studies on phenotypic variation in VWM are scarce. Chapter 2 provides data collected and analyzed from our VWM patient database, the largest cohort so far, to gain more systematic knowledge on the clinical variation in VWM. Data were collected on prevalence and characteristics of subgroups of patients defined by age of onset.

The large clinical spectrum of VWM was confirmed in the dataset. The VWM disease spectrum consists of a continuum of phenotypes with extremely wide variability. The spectrum continues to expand on both extremes suggesting that even more extreme phenotypes are currently missed. The division in certain age of onset groups is arbitrary, though clinically useful. Most patients present in childhood. Disease severity clearly correlates with age at onset of symptoms; the younger the first neurological signs appeared, the more severe the disease course is with a higher sensitivity to stress. We suspect that especially adult onset, mild variants of VWM have largely been underdiagnosed, because of the less typical presentation and the lack of awareness under adult neurologists. More and larger follow-up studies will further contribute to a more representative description of the clinical spectrum in VWM patients.

MALE-FEMALE DIFFERENCES

In VWM the male-female ratio is not equal for patients of all ages. Among adult onset VWM patients, a predominance of females has been observed.[18] This is unexpected for an autosomal recessive disorder. Labauge and colleagues hypothesized that the reason for the imbalance in ratio between males and females is that with mild mutations, females are more prone to disease presentation, while more males remain asymptomatic.[18]
In chapter 2 and 5 we studied male-female differences. We also found a higher number of VWM teenage and adult females, with a trend for higher survival rates and less rapid loss of ambulation among females; so a milder disease than males. However, if males would tend to have a more severe disease, one would expect more males in the younger age at onset groups, which we did not find. It is important to note that in general, male have a shorter life expectancy than females and at all ages more men than women die. Balsara et al.[19] suggest the existence of a male vulnerability factor, attributed to a complex interplay of factors including acquired risks, health-reporting behavior, illness behavior, health care utilization as well as an underlying biological difference explaining the general male disadvantage in life expectancy.[19] However, in infantile and early childhood onset VWM, these gender differences are less pronounced. Larger numbers of patients are required to find out whether the general male:female imbalance explains the imbalance in VWM or whether the male disadvantage is more prominent in VWM than explained by the general 'life expectancy gap'.[19,20] A factor that could contribute to the observed male-female imbalance is that females with mild variants of VWM are more readily diagnosed because of the ovarian failure.[18] It is possible that in the category of mildly affected individuals who exhibit only subtle neurological signs, this feature advances diagnosis in woman, while the diagnosis in equally mildly affected males is missed.

We are aware of shortcomings of our clinical variation studies. Retrospectively collected data are of lower quality than prospective data. Our study on clinical variation is the largest described VWM cohort, but still the numbers are often small in the subgroups. The characteristics of our phenotypic variation study with its international and multi-institutional nature with physicians and families filling in the questionnaires, both having a different background, may all hamper a truly objective evaluation of the clinical course in VWM patients. Missing data are often not random, for example missing data on early childhood in adult patients. It is possible that over time a selection bias occurred as VWM was initially recognized as a disease in childhood with a possible underestimation in the older age of onset groups. For future studies on clinical variation, a study at a larger scale and with longer follow-up of VWM patients could give more insight into the true clinical variation. If treatment would become available and case-control studies would be unethical, a well-documented, large database of historical controls is mandatory. So, the Amsterdam VWM database is a long-term project with new information being entered.

MRI CHARACTERISTICS IN VWM

Before DNA testing was available, the diagnosis of VWM was made by clinical and MRI criteria. Nowadays, using genetic analysis as the 'gold standard', the proposed MRI criteria have 95% sensitivity and 94% specificity.[1,7,9]

MRI in early stages of VWM disease

The MRI criteria are suitable to diagnose cases with typical presentation, but are not suitable for identifying unusual MRI variants like the most severe and the mildest variants.[7,8] Additionally, MRI criteria may not be met in the earliest stages of the disease. In chapter 3 we studied the MRI characteristics of the early stages of the disease, preceding the stage of rarefaction and cystic white matter degeneration. The study showed that in early stages of VWM, MRI does not necessarily display diffuse cerebral white matter abnormalities and also rarefaction or cystic degeneration is not necessarily present. All patients included in the study had confluent and symmetrical abnormalities in the periventricular and bordering deep white matter. In young patients, myelination was delayed. The inner blade of the corpus callosum was affected in all patients. A conclusion of this study is that if the MRI abnormalities do not meet the criteria for VWM, it helps to look at the corpus callosum: if the inner blade is affected, VWM should be considered.

Restricted diffusion

Only a few studies mention the results of diffusion-weighted imaging (DWI) in VWM.[7,21-23] On diffusion-weighted images, the rarefied and cystic white matter demonstrates an increased diffusivity related to highly expanded extracellular spaces.[7,8,21-24] However, areas of restricted diffusion are found in some patients.[8,23,24] It has been suggested that the restricted diffusion in VWM reflects acute brain tissue degeneration or acute demyelination[23,24], but the exact histopathological characteristics underlying restricted diffusion remain unknown.

In chapter 4 the occurrence of restricted diffusion in vanishing white matter was investigated. Areas with decreased apparent diffusion coefficient values were found in the U fibers, cerebellar white matter, middle cerebellar peduncle, pyramids, genu or splenium of the corpus callosum, and posterior limb of the internal capsule. All are relatively spared regions in VWM.[9,15,25,26] Patients showing restricted diffusion were younger and had shorter disease duration. Histopathologic analysis of a brain slice revealed that the regions with restricted diffusion had a higher cell density and did not show tissue degeneration. These findings are in agreement with the observation that relatively spared regions, which show diffusion restriction, have the highest cell density in VWM.[26]

Our findings added to the knowledge on MRI in early stage of the disease and DWI findings in VWM. A still difficult problem is posed by MRIs of (young) adults that show diffuse white-matter abnormalities without rarefaction or cystic degeneration.[7-9,27,28] A valid question is whether in such cases mutational analysis of the *EIF2B1-5* genes should be performed. The contribution of the known biochemical markers glycine (ratio of CSF to plasma)[29] and asiolotransferin[30,31] has

not been evaluated in such cases.[7,8,28] Our findings suggest that involvement of the inner blade of the corpus callosum helps in the decision. Meoded and colleagues[32] propose adding spinal imaging, as they found involvement of the cervical posterior spinal tracts and mild global spinal cord atrophy in a single patient. It is at present unclear if these findings are sufficiently specific for VWM to be of help. Histopathology findings show that in the spinal cord is only partially affected.[1,9,15,16,25]

GENOTYPE-PHENOTYPE CORRELATIONS

The explanation for the wide phenotypic variation in VWM is complex. Certain mutations are consistently associated with a mild or severe phenotype.[11,27,33,34] On the other hand, environmental and/or other genetic factors than the eIF2B mutations appear to determine at least part of the phenotype, as within families phenotypic heterogeneity has been reported.[7-9,11,17,27,35]
In chapter 5 we looked at the correlation between genotype and phenotype and addressed the question whether the clinical phenotype of compound-heterozygous patients is determined by the mildest mutation, the most severe mutation or by both. We selected the three most frequent mutations in *EIF2B5*: p.Arg113His, p.Thr91Ala and p.Arg339any, associated with a mild, mild to intermediate, and severe phenotype, respectively.
Our findings demonstrate that patients homozygous for p.Arg113His have a milder disease than patients compound-heterozygous for p.Arg113His and patients homozygous for p.Thr91Ala. Patients with p.Arg113His/p.Arg339any have a milder phenotype than patients with p.Thr91Ala/p.Arg339any.
These findings indicate that the phenotype is not determined by the most severe or mildest mutation alone, but by the effect of both mutations. This conclusion is important for clinicians and genetic counsellors providing information to patients and families.
One should be careful, however, with definitive predictions for new patients. Further studies on larger groups of patients would make conclusions more definitive. In view of the many different mutations increasing the study scale is conditional for better insight into genotype-phenotype correlation.

eIF2B DYSFUNCTION

Mutations in eIF2B affect eIF2B function in diverse ways.[36-39] When tested in patient-derived lymphoblasts and fibroblasts mutations were reported to decrease eIF2B activity as guanosine exchange factor (GEF).[40] The severity of the decrease was suggested to correlate with the clinical severity, although later data showed inconsistencies in this correlation.[40,41]
In patients' lymphoblasts and fibroblasts, the decreased eIF2B activity was not found to affect the rate of global protein synthesis, before, during or after stress or cell proliferation and survival.[38,47,48] These observations suggest that basal eIF2B activity by itself may not or not straightforwardly explain the disease.[8,17] Assessment of eIF2B activity in patient-derived cell lines has

been proposed as a tool in the diagnosis of VWM[41], but considering the above observations, it is questionable whether eIF2B activity as measured in cells reflects anything significant regarding the disease and whether it can be used as a marker of the disease or of disease severity.
In chapter 6 we focused on the functional effects of selected VWM mutations by co-expressing mutated and wild-type subunits in human cells and combined these studies with measurement of the GEF activity of eIF2B in patient derived cells with the same mutations. Our findings showed that the observed functional effects are diverse, including defects in eIF2B complex integrity, binding to the regulatory alpha-subunit, substrate binding and GEF activity. Strikingly, some of the studied mutations causing severe disease did not alter eIF2B function in the tested parameters. Some even resulted in elevated GEF activity.
Measurement of eIF2B GEF activity in patient-derived lymphoblasts and fibroblasts as published has therefore limited value as a diagnostic test. It is at present unclear if the protocol used for measurement of GEF activity reflects physiological conditions, if the used patient-derived cell types are the right system for such measurements, or if the disease is altering eIF2B function in another way than measured.

An experimental set-up with cultured brain cells derived from VWM patients would be ideal to study effects of VWM mutations on cells from the most affected organ, the brain, to study for example GEF activity. This could also be done with cells from the ovaries and other affected organs in de severe antenatal forms.
A mutant mouse model provides an alternative for this option. A mutant mouse model for VWM has been developed[42-44], but this mouse lacks a clear phenotype. Although interpretation of results obtained from mouse models for human diseases is hampered by species differences, a representative mutant VWM mouse would allow studies on all types of cells at all stages of the disease. Another option would be to use VWM patient derived induced pluripotent stem cells that can be differentiated into neural cells, especially oligodendrocytes and astrocytes. Such cells could be more suitable for the study of effects of mutant eIF2B on cell functions, including GEF activity.

The total amount of eIF2B per cell and eIF2/eIF2B ratio have been shown to vary between different tissues.[45] The eIF2B concentration is the rate-limiting step in mRNA translation velocity. Different expression levels of eIF2B and differences in eIF2/eIF2B ratio in brain cells or other cell types may influence the sensitivity of the translation initiation process to the control exerted by phosphorylation of eIF2α in such cells. The total amount of eIF2B and the
eIF2/eIF2B ratio have not been determined for different human brain cell types and for cells in different regions of the brain, while such differences may contribute to the selective vulnerability of the white matter and white matter regions in VWM. Studies in a mutant mouse model may contribute to the insights on this subject.

Several authors have investigated the hypothesis that the decreased eIF2B activity might impair the cellular stress response and improperly activate the unfolded protein response (UPR).[46-48] These suggestions can be confirmed in mutant mice with VWM.

Aberrant control of translation of specific mRNAs has been put forward as possible effect of eIF2B dysfunction.[49-51] Decreased eIF2B activity leads to reduced general rates of protein synthesis, but to increased synthesis of some proteins, depending on the structures of the 5'untranslated region of the mRNA. It could be that such proteins are central in the disease mechanisms of VWM. It is also possible that eIF2B serves additional functions, apart from those in translation initiation[8,52] and it could be that a defect in those functions in fact cause the disease VWM.

Until now, research on VWM has been most of all hypothesis-driven. With this approach small pieces of the VWM puzzle have been laid, as discussed above. However, we are far away from understanding the big VWM picture. Further research should be more open, not based on an a priori hypothesis. With a more open mind we may start to begin to gain better insight into the pathophysiology of VWM. Such insight is essential for developing treatment.

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